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# A fluidic fiber platform modified for the selective extraction and on fiber fluorescence detection of proteins and nucleic acids

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A FLUIDIC FIBER PLATFORM MODIFIED FOR THE SELECTIVE  
EXTRACTION AND ON FIBER FLUORESCENCE  
DETECTION OF PROTEINS  
AND NUCLEIC ACIDS

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A Thesis  
Presented to  
the Graduate School of  
Clemson University

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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science  
Chemistry

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by  
Natasha Khan  
May 2014

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## ABSTRACT

Solid phase extraction has become a popular tool for analytical method development as a sample preparation technique. Solid phase sorbents can generally extract analytes based on polarity or selectively based on highly specific interactions between the analyte and an immobilized surface molecule. Biomolecular recognition based solid phases utilize antibodies, nucleic acids, and proteins as a secondary affinity layer on a solid phase support for selective analyte extractions and have been used for sample preparation, purification, separations, bioassays, and molecular diagnostics.

Polymer supports for solid phase extraction are desirable for their low cost, abundance, chemical inertness, and pH stability. This thesis reports the use of capillary-channel polymer (C-CP) fibers as a solid support modified for selective extraction and on fiber fluorescence detection of nucleic acids and proteins. The polymer fibers are low cost and their unique shape allows them to be easily adapted into a fluidic fiber based micro spin column configuration for a small scale, inexpensive, low-tech, and easy to use device. Platform performance was successful as demonstrated by modifying the polymer surface with neutravidin via adsorption to tether specific biotinylated analyte recognition moieties. Fluorescence resulted from either hybridization of a complementary fluorophore labeled probe to nucleic acids or conjugation of a fluorophore to the analyte protein.

On fiber fluorescence detection of nucleic acids and proteins was successful with minimal non-specific binding of proteins and nucleic acids to the polymer surface. Two sequence specific oligonucleotides were added and probed either separately or

simultaneously on the fibers; the fluorescence intensity increasing with the amount of oligonucleotide added, leveling off at 100 pmol. Fluorescence signal never saturated for small volume additions (10ul) up to 100 pmol of added oligonucleotide. The LOD was calculated to be 100s of fmol for all experiments. For selective extraction of proteins, protein recovery was similar for both C-CP fibers and streptavidin conjugated magnetic microbeads. Adsorbed neutravidin did not irreversibly bind to the C-CP fiber surface and was partially removed in the presence of .01% Tween-20.

C-CP fibers and spin columns are inexpensive, easy to use, and can be integrated as a solid phase extraction sample preparation step for small scale bioassays or molecular diagnostics.



## DEDICATION

I would like to dedicate this thesis and the work herein as well as my journey through graduate school to my Father in Heaven and His son Jesus Christ. For it was through their example that has made me the person I am today. To my husband, Mark Monson who believed in me even when I doubted myself. Thank you for your love and support. To my mother, JoAnna Khan and sister, Christina Shiner for allowing me to focus on my education. To all the wonderful people I have befriended over the years here in South Carolina. Also, to all those involved in the McNair Scholars program at Westminster College who helped me get to graduate school.

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## CHAPTER ONE

### INTRODUCTION

The ability to analyze the composition, quantity, and identity of certain molecular and biomacromolecular compounds has led to advancements in therapeutics<sup>[1-3]</sup> and medical diagnostics<sup>[4-8]</sup>. Direct analysis can be problematic due to interfering sample matrix particles and molecules that can suppress or enhance the analyte signal. Samples that contain trace amounts of analyte are also not suitable for direct analysis because they can be below the detection limit for most analytical instruments. Solid phase extraction has become a popular tool to analyze compounds from a variety of complex mixtures and biological matrices such as groundwater<sup>[9-12]</sup>, urine<sup>[13, 14]</sup>, and whole blood<sup>[15, 16]</sup>. Solid phase extraction works by extracting out the analyte of interest onto a solid phase sorbent that has favorable interactions with the analyte. It has been widely used in analytical method development as a sample preparation step, making the analyte of interest conducive for downstream application. Depending on the downstream application the analyte can be purified, isolated, or pre-concentrated. For example, water purification uses solid phases when extracting drugs<sup>[17]</sup>, pesticides<sup>[18]</sup>, and metals<sup>[19]</sup> from ground water. Silica particles and ion exchange resins have been used to extract DNA before downstream PCR analysis because the salt and protein content found in DNA samples (from cell lysate) can inhibit enzyme amplification<sup>[20-22]</sup>. Solid phase extraction has been used as a sample purification and analyte isolation prior to ESI and MALDI mass spectrometry detection because biological fluids are composed of many ions that can interfere or complicate mass spectrometry detection. The salts cause ion

suppression as well as forming adducts that can add more peaks which complicate analysis<sup>[23-26]</sup>. Pre-concentration has been used in both the environmental and forensics fields for trace analysis of pesticides from environmental samples<sup>[11]</sup> and for DNA from forensic samples<sup>[27, 28]</sup>.

Solid phases come in a variety of materials and formats depending upon the application. Conventional solid phase sorbents packed into cartridges, columns, and disks<sup>[29]</sup> are more generally selective and extract the analyte of interest based on its polarity and electrostatic interactions between it and the sorbent. These phases are classified into different modes such as normal phase, reverse phase, ion exchange, and mixed mode extraction<sup>[30]</sup>. Modified silica is the most used for all modes because it is easily modified to alter the polarity with different polar and non-polar functional groups such as C-18, C-8, CN, and phenols<sup>[30]</sup>. However, silica has disadvantages such as low pH working range and chemical instability from hydrolysis of the bonded chains, and the effects of the unreacted silanol groups<sup>[30, 31]</sup>. This led to carbon based solid phases which had better chemical stability compared to silica. Carbon solid phases such as carbon black and porous graphitized carbon were shown to have some degree of polarity and non-polarity which acted as more of a mixed mode and would extract out polar, non polar, and ionic substances<sup>[31]</sup>. However carbon phases irreversibly bound polar analytes which resulted in poor recovery<sup>[31]</sup>. Porous polymer resins<sup>[32]</sup> such as polystyrene divinylbenzene also have better chemical stability compared to silica however they have to be pretreated with polar solvents before analyte extraction from aqueous solutions because the polymer was hydrophobic. This led to functionalized polymer solid phases



linked with hydrophilic functional groups to allow aqueous solutions to make better contact with the resin<sup>[32]</sup>.

Silica, carbon, and polymer sorbents are only generally selective and can include other co-extracted interferences. This has led to method development that includes a two step solid phase extraction and downstream separation to fully isolate the analyte, or multiple elution steps to get rid of the other co-extracted interferences<sup>[33]</sup>. Also co-extracted interferences can also decrease binding capacity, which can be detrimental in trace analysis. For example, DNA and RNA have been extracted on both ion exchange and silica sorbents<sup>[34]</sup>. The negatively charged phosphate backbone on nucleic acids can be exchanged on an anion exchange phase or hydrogen bonding of nucleotide bases on a silica phase. However, proteins also bind to the sorbent and have to be removed in a first elution step with ethanol.

Extraction efficiency is due in part to the affinity of the analyte to the solid phase sorbent. The introduction of selective sorbents can enhance extraction down to just a single analyte or a similar group of analytes. This has led to the development of selective solid phases based off of molecular recognition such as molecular imprinted polymers (MIP) and immunosorbents. MIPs operate on the principle of using template molecules bound to functional monomers that are polymerized around it to form cavities in the shape and size of the template molecule that mimic the actual natural binding sites. The template molecule is then extracted out of the cavity. They are desirable because the template holes are in the shape and size of the molecules such as small ionic molecules and even proteins, which give them their selectivity. They have been used for

environmental analysis of pesticides<sup>[35]</sup>, and drugs<sup>[35, 36]</sup>. However MIPs suffer from disadvantages. One major one is template bleed through, meaning the template still remains embedded in the polymer after all the washes to extract it out of the cavity<sup>[35]</sup>. This disadvantage has been circumvented by the use of a similar analogue as the template so there will not be bleed through. MIP have also been used for protein extraction<sup>[37]</sup> but has disadvantages because of the unstable 3D conformations of proteins brought on by random motion as well as conformational changes which are dependent upon the ionic strength, solvent, and pH. Immunosorbents are also based on molecular recognition and use antibodies that have been raised against an antigen for selective antigen extraction<sup>[12]</sup>. Immunosorbents have found the most use in biological separations<sup>[38]</sup>, but have also been adapted for the extraction of small molecules in environmental<sup>[12, 39]</sup> and food samples by using specific antibodies directed against those antigens<sup>[12]</sup>. Nucleic acids<sup>[40]</sup> and proteins<sup>[41]</sup> have also been used as biomolecular recognition ligands for solid phase extraction and have found application in areas such as molecular diagnostics and bioassays<sup>[7]</sup>.

Solid phases also come in a variety of configurations depending on the need, instrumentation, available resources, and technical skill level required. One such configuration is the microbead. Biological separations and extractions were revolutionized after the invention of the microbead in 1976 and become more popular when they were made magnetizable<sup>[42]</sup>, which made it easier for sample handling and did away with instrumentation unlike agarose and dextran modified beads which had to be pelleted by centrifugation. The magnetic microbeads have been made with diameters

from tens of nanometers to hundreds of micrometers and have been used in bioassays as solid phase substrates<sup>[43-46]</sup>. The microbead configuration allowed for smaller scale solid phase extraction and affinity purification with the potential for parallel and high throughput applications compared to the traditional solid phase extraction configurations such as columns, cartridges, and disks which required larger volumes that increased sample processing time resulting in less throughput. The beads are scalable and can use smaller volumes of hundreds of microliters because the beads in conjunction with smaller volumes increase the available surface area available per volume for binding. Beads are sold either pre-coupled or modified “in house” with specific ligands such as protein A and G<sup>[47, 48]</sup> as well as other antibodies<sup>[48]</sup> which have been used in immunoassays and purifications<sup>[49, 50]</sup>, along with streptavidin<sup>[43]</sup>, glutathione<sup>[47, 49, 51, 52]</sup>, nickel<sup>[51, 52]</sup>, and even conventional solid phase functional groups for other small scale purifications or bioassays<sup>[53]</sup>. There is also polystyrene beads which allows for “in house” customization<sup>[54, 55]</sup>.

Microtiter plates made from various polymers (polystyrene, acrylic, PMMA) are another configuration that have been used as solid supports for selective extraction. One example is functionalizing the microtiter wells with either antigen or antibody to test for the presence of certain antibodies and antigens in serum. When the body is infected with a particular antigen, such as a virus or a particular allergen, it builds up immunity towards it by producing antibodies. To test for the presence of these antibodies, the antibodies need to be extracted out of the serum. The antibodies are extracted onto the immobilized antigen on the well surface and then probed with a secondary labeled

antibody for detection. For example, enzyme linked immunosorbent assay (ELISA) is a pre-screening test where diluted blood serum is applied to an immunosorbent or antigen modified well in a microtiter plate<sup>[56]</sup>. The immobilized antibody from the serum sample is then probed with a secondary antibody linked to an enzyme, and upon addition of a specific enzyme substrate, the solution in the well will change color if the particular serum antibody was present. The microtiter plate configuration allows for semi-quantitative readout from the well, simple sample application, surface modification, and the ability to run parallel assays. Plus, the increased sensitivity of enzyme/substrate detection makes it suitable to detect very small amounts of antibody.

Microfluidics has also found multidisciplinary use in fields of chemical synthesis<sup>[57]</sup>, fluid delivery<sup>[58]</sup>, and solid phase extraction<sup>[59]</sup>. Each field has different motivations for microfluidics. However, all fields find potential for automization, integration, and portability in these miniaturized, self-contained devices. This has led to a whole area of research dedicated to the miniaturization of conventional bench top procedures onto a single chip for a total analysis system<sup>[60]</sup> known as “lab-on-a-chip.” There has been an ongoing effort to develop simpler designs in order to make more easily used diagnostic devices to become more widespread making their way from centralized laboratories to point of care facilities such as clinical facilities and hospitals. Ultimately, the ideal goal is to have a “plug and play” device that can be used for as a total analysis system. Part of that total analysis is to incorporate solid phase extraction into the chip. There has been research done that has incorporated silica beads, sol-gels, and polymer monoliths into microchannels for the extraction of nucleic acids<sup>[6, 34, 61-71]</sup>. This has allowed for less

sample handling which reduces contamination, and has expedited analysis time due to minimal sample volumes needed as well as low elution volumes for nucleic acid enrichment. There has also been research done where amplification is incorporated on the chip using various temperature cycling strategies such as miniature heaters, chemical reactions, as well as just using isothermal amplification<sup>[70, 72-75]</sup>. Integrating extraction and amplification on-chip reduces the risk of sample contamination and degradation of the nucleic acids.

There has been an ongoing effort to also develop fluidic type devices using low cost, abundant materials that require very little technical skill and instrumentation. In a low resource setting or on-site testing there will most likely not be a skilled technician or instruments needed to handle sample processing and analysis so a practical alternative would be a rapid, integrated, and portable design with direct extraction from biological fluids and visual detection (i.e. colorimetric detection) preferably, on a cheap solid support. There has been research using paper or nitrocellulose membranes for microfluidic chips and lateral flow assays for this purpose coupled with probes for colorimetric detection<sup>[76-83]</sup>.

There has already been some research using electrospun fibers as well as polyester, cotton, silk, and cellulose thread as material for solid phase extraction and fluid transport<sup>[84-103]</sup> as an ongoing effort to design simple, cheap, low-tech fluidic devices. The fiber platform used in the following studies is made possible by production of capillary channel polymer (C-CP) fibers that are made from textile materials extruded from a variety of low cost polymers that include polypropylene (PP), polyester, and

nylon. The shape and geometry of the individual fibers is unique and determined by their extrusion; we have been working with PP fibers extruded with a series of eight open channels. Although PP is highly hydrophobic which does not easily wick fluid, however, when packed together the interdigitation of shaped fibers creates micron-sized channels with efficient fluid transport and high surface area that still promote fluid wicking for aqueous solvents when the fluid is passed through by some force, which for us is centrifugation. As stated before, the type of sorbent used for solid phase extraction depends on the resources available and the chemistry needed. The PP fiber surface is hydrophobic so its surface chemistry is not easily altered by pH and has minimal binding via electrostatic interactions to other predominantly charged species in biological sample matrices as well as minimal interactions with the extremely negatively charged backbone and hydrogen bonding of nucleic acids. Another advantage of the PP hydrophobic material has shown to be easily modified via protein adsorption<sup>[104]</sup> which works well to use them as a simple fluidic platform. C-CP fibers have already demonstrated platform performance being used in other applications for protein<sup>[104, 105]</sup> and metal extraction<sup>[106]</sup>, and purifying proteins by extracting them from high salt solutions for MALDI analysis<sup>[107]</sup> and have extracted analytes from complex matrices such as urine and saliva<sup>[104]</sup> and lysate<sup>[108]</sup> Similar shaped fibers have been used as packing materials for column chromatography, where their fluid transfer properties ameliorate high back pressures otherwise required for large-scale preparative column chromatography<sup>[109-112]</sup> Previous work done using PP C-CP have minimized non-specific

adsorption and have successfully and selectively extracted proteins from buffer and lysate if the proteins are in the presence of Tween-20<sup>[108]</sup>.

The fiber surfaces can be easily modified via protein adsorption for specific functionality and can be used as a highly selective solid support for a range of affinity capture assays and selective solid phase extractions. The fibers in the following chapters were modified with neutravidin via adsorption for specific recognition of biotinylated molecules. The avidin-biotin interaction is the strongest non-covalent binding interaction in nature with a  $K_d$  on the order of  $10^{-15}$  M. Neutravidin, is the deglycosylated form of avidin, and deglycosylation helps minimize non specific binding unlike avidin and raises the isoelectric point to a more neutral value (6.3) to give it good solubility in aqueous solutions. Biotin labeled molecules are good for protein labeling because biotin is small enough not to disrupt the conformation or alter the proteins activity. Most proteins and nucleic acids contain or can be easily modified with primary amines or carboxylic acid groups that can be easily labeled with biotin. This highly stable interaction between biotin and avidin and its similar analogues has been exploited and used in various assays where the solid phase is modified with an avidin-like protein in order to bind biotin labeled molecules. Using this, the following studies in this thesis reports a proof of principle demonstration of a fluidic fiber platform for selective extraction of biomolecules by modifying the fibers with NAv via protein adsorption followed by the addition of a specific biotinylated analyte recognition moiety.

In the following chapters, individual microscopic PP C-CP fibers are bundled and pulled into a spectroscopically clear (visible spectrum) 0.8 mm capillary FEP tubing that was then cut into 0.5 cm pieces and loaded into the narrow end of a 200  $\mu$ l micro-pipet tip. When fiber surfaces are modified with molecular recognition elements, each fiber-filled tip becomes a platform for molecular recognition solid phase extraction or assays. The pipet tip acts as a reservoir for analyte addition. Results reported here describe application of neutravidin modified PP C-CP fibers to extract and detect nucleic acids and proteins. Chapter 2 goes over making a selective solid phase modified with biotinylated [dT]<sub>20</sub> for mRNA extraction that is also coupled with on-fiber fluorescence detection where the mRNA is probed with complimentary fluorescent oligos. Chapter 3 discusses the application of PP C-CP fibers for pull-down assays and comparing protein recovery between fibers and streptavidin conjugated magnetic microspheres as well as on-fiber fluorescence detection of the protein of interest. This new fiber configuration is distinguished by its simple low-tech design, speed, flexibility, and low cost.



## CHAPTER TWO

### AN INTEGRATED FLUIDIC PLATFORM FOR EXTRACTION AND DETECTION OF SIMULATED mRNA ON CAPILLARY-CHANNELED POLYMER FIBERS

With the completion of whole genome sequencing, nucleic acids have found use as biomarkers in a variety of applications such as molecular diagnostics<sup>[113-124]</sup> forensics<sup>[125-127]</sup> gene expression profiling<sup>[128-131]</sup> detection of biowarfare agents<sup>[132]</sup> and environmental analysis<sup>[133]</sup>. Depending on the application, nucleic acids have been generally extracted onto silica and anion exchange solid phases by hydrogen bonding and electrostatic interactions or by biomolecular recognition via complimentary base pairing of a target nucleic acid strand to an immobilized nucleic acid modified solid support. The widespread utility of nucleic acid detection is in part due to the development of inexpensive, simple, rapid, low-tech, microfluidic devices for easier sample preparation by solid phase extraction of nucleic acids. Molecular biology has revolutionized the field of bacterial diagnostics as a rapid alternative over bacterial culture using the pathogens own DNA and RNA as biomarkers to identify and make definitive diagnosis of bacterial species and its resistance in only a few hours.

A rapid, low-tech and low cost polymer solid support was modified for selective extraction and highly sensitive detection of simulated mRNA using a novel fiber-based microfluidic platform made from low cost textile fibers. The simulated mRNA was designed with oligonucleotide sequences coding for a region of either TNF- $\alpha$  or  $\beta$ -actin specific mRNA sequences including a [dA]<sub>20</sub> tail as a stand-in for the complete mRNA.

Extraction and detection was integrated on the fibers with downstream analysis that consisted of on fiber fluorescence detection of the simulated mRNA. Integration of extraction and detection is desirable because it minimizes sample handling, which can lead to sample contamination and degradation. Although visual detection is practical for those areas, it is not as sensitive as alternative detection strategies. A laboratory in a hospital or clinicians office has moderately skilled staff that could still achieve rapid processing using simple low-cost designs, but also be able have access to instrumentation for better sensitivity using fluorescence, electrochemical, or chemiluminescence detection<sup>[43, 134-145]</sup>.

## **Experimental Section**

### Materials

Neutravidin (NAv) was purchased from Thermo Scientific and diluted to 60 ppm into phosphate buffer saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) for all experiments. For a blocking reagent, previous research shows adding Tween-20 (Rockland) to PBS (PBS-T) at a final concentration of .01% (v/v) sufficiently reduced non-specific binding<sup>[108]</sup> All DNA oligonucleotides were purchased from IDT (IA) and diluted into PBS-T. Biotinylated [dT]<sub>20</sub> was diluted to 1 uM in PBS-T for all experiments.

The PP fibers (Eastman Chemical) were constructed into “tips” by using protocols referenced elsewhere but with slight modifications<sup>[104]</sup>. Briefly, 470 PP fibers were manually wound using a rotary counter and rinsed with 95°C water, MeOH, ACN, and milliQ water. Once the fibers dried, the fibers were pulled into 30 cm long, 0.8 mm

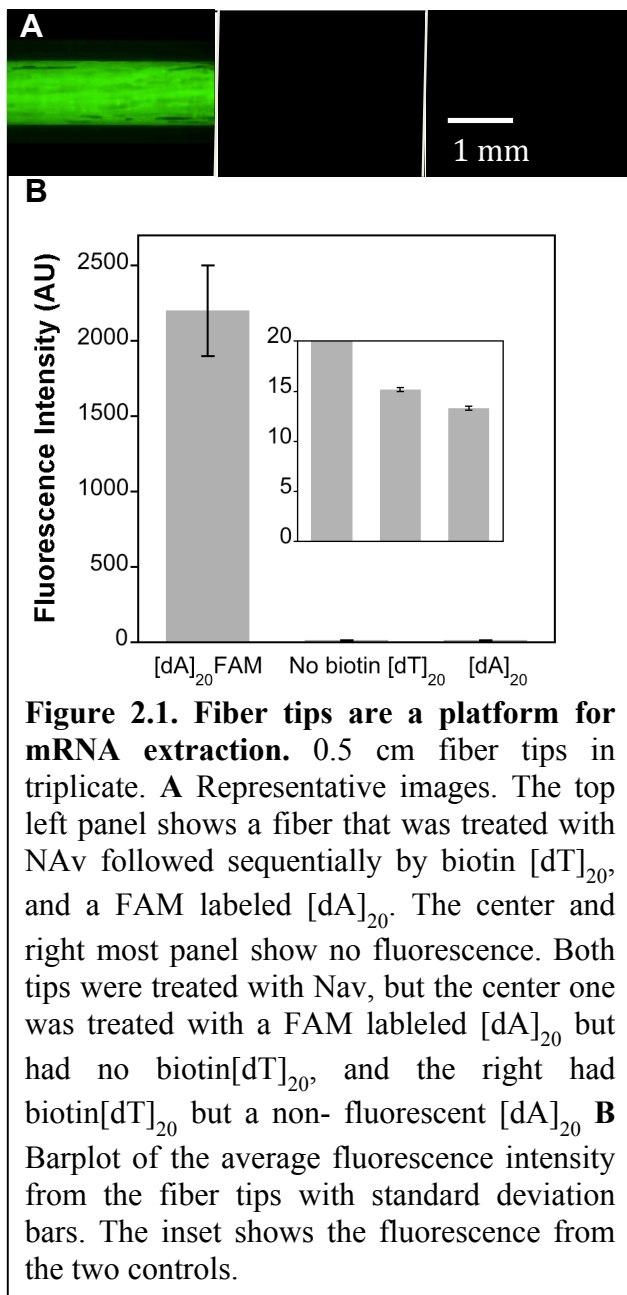
optically clear (visible spectrum) fluorinated ethylene propylene (FEP) capillary tubing (Cole-Parmer). Tips were created by cutting the bundle down to individual 0.5 cm pieces leaving a 6 mm gap to fit it to the end of a 200  $\mu$ l micropipette tip that was then cut and fitted to the end of a 1 ml micropipette tip that acted as the reservoir for fluids to be passed through the fiber spin columns during centrifugation. A 15 ml tube collected the effluent. The apparatus was completed by fitting the tip and reservoir micropipette tip assembly through a hole that was formed by drilling into the top of the 15 ml tube lid.

#### Experimental outline

Each functionalization step (including washes in between each reagent addition) for all experiments used 100  $\mu$ l of volume (except for small volume experiments which used 10  $\mu$ l) and spun at 220 X g for 1 min at 21°C. PP C-CP fiber tips were modified by serial addition of NAV, biotinylated [dT]<sub>20</sub>, and either a fluorescent and non-fluorescent [dA]<sub>20</sub>. All other experiments for on fiber detection of simulated mRNA were done by similar modification with NAV, and biotinylated [dT]<sub>20</sub>, but with serial addition of simulated mRNA and complementary fluorescent labeled probes (500 nM). All reagents used were kept on ice until needed.

#### Data acquisition and analysis

With the fibers still packed into the tubing, the fluorescence from the fiber tips was detected and imaged at 2X magnification (2X/.08 NA UPlan) using an epiillumination microscope (Olympus IX71) connected to an Orca-ER (Hamamatsu) CCD camera and Xe arc lamp excitation source. The fluorescence for fluorescein amidite (FAM) and Texas Red labeled oligos was detected using 494 ex/ 531 em filter and 575 ex/624 em



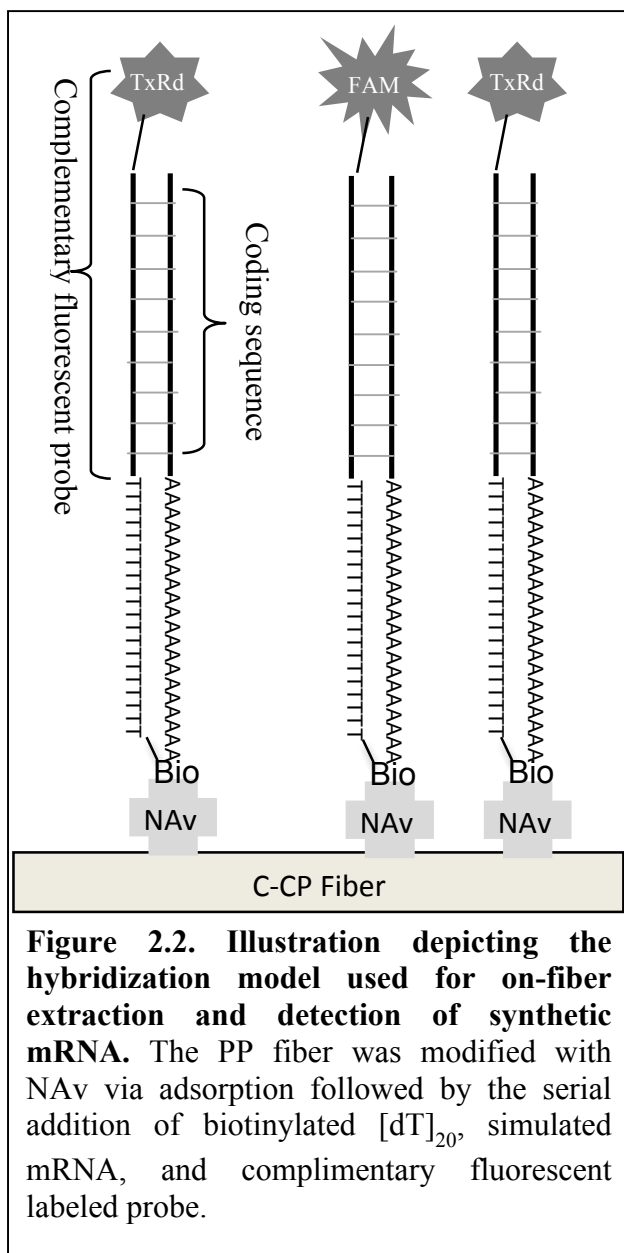
filter respectively. The fluorescent fiber images were collected and quantified using Slidebook 5.0. Each experiment used 3 fiber tips and the average fluorescence intensity from each single fiber tip was averaged over the triplicate tips. All plots were generated using Kaleidagraph 4.1 (Synergy Software).

## Results and discussion

Solid phase extraction can be made more selective by modifying the solid support with specific analyte recognition moieties. A model system was first constructed to generally extract mRNA on the fibers. Most mRNA found in mamillian cells are tagged with a

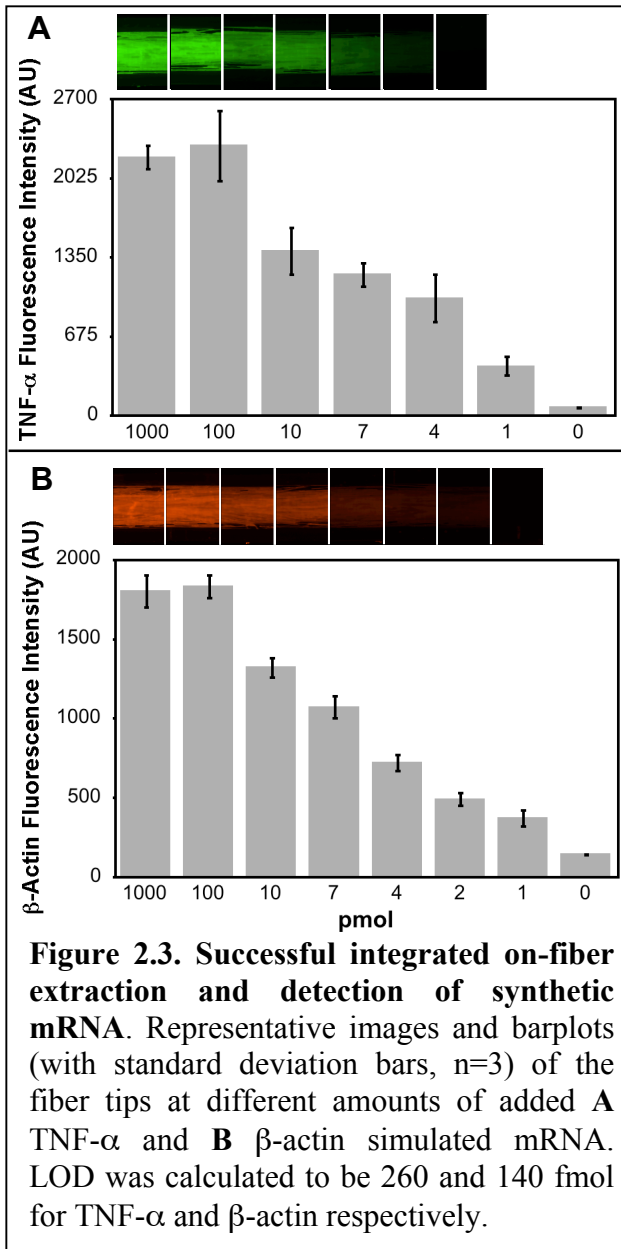
polyA tail. This made it easy to design and test a model system that used a biotinylated  $[dT]_{20}$  tethered onto the C-CP fibers via NAv to extract the mRNA by  $[dA]_{20}/[dT]_{20}$

hybridization. A FAM labeled  $[dA]_{20}$  was then added as a fluorescence indicator for  $[dA]_{20}/[dT]_{20}$  hybridization.



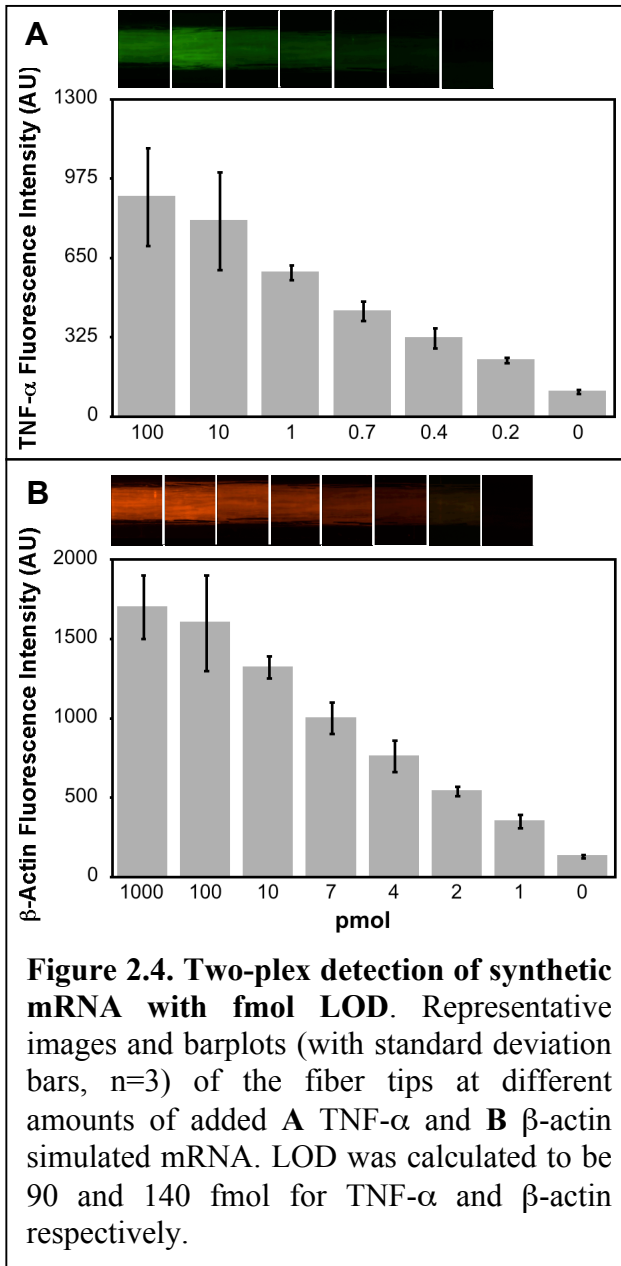
extraction.

As shown in **Figure 2.1** successful hybridization was achieved with significant fluorescence associated with fiber tips treated with NAv, biotinylated  $[dT]_{20}$  and the  $[dA]_{20}$  fluorescent labeled oligo, but not with tips in the absence of biotinylated  $[dT]_{20}$ . The data also shows very minimal non-specific binding of the FAM labeled  $[dA]_{20}$  compared to a control where a non-fluorescent  $[dA]_{20}$  was used which suggest that DNA oligos (in the presence of PBST) do not adsorb to the PP fiber surface also suggesting the presence of adsorbed NAv on fiber surface, and demonstrates that adsorbed NAv can be used to tether biotin-linked recognition elements to the fibers as a platform for mRNA



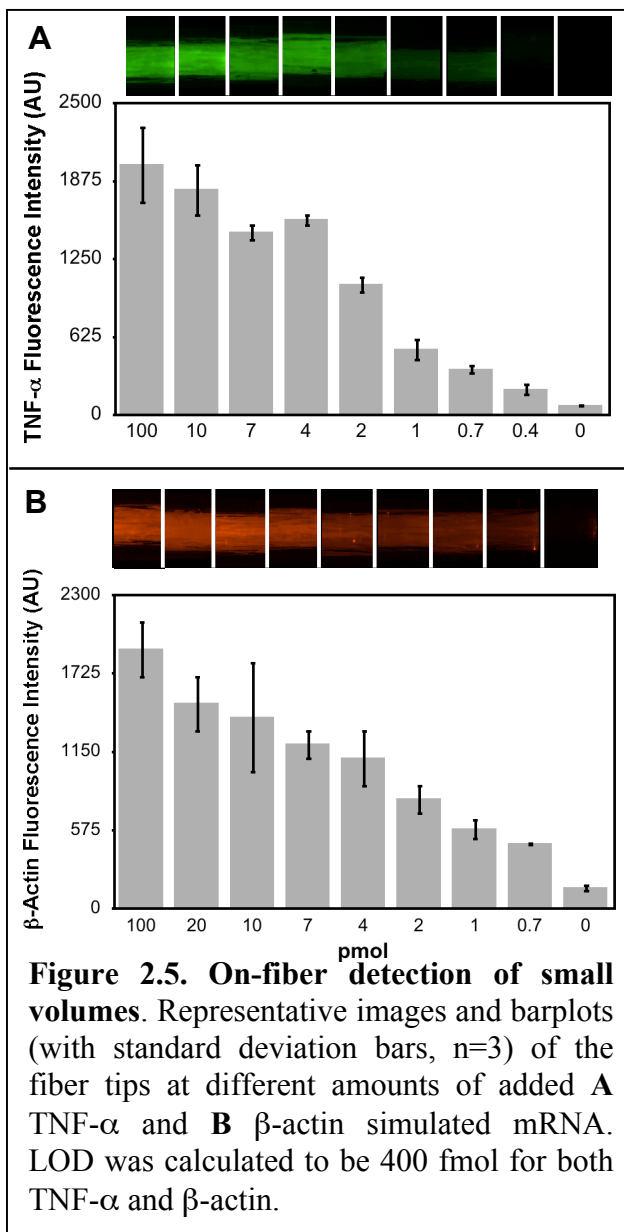
mRNA extraction and detection was then integrated on the fibers by probing the simulated mRNA with a complementary fluorescent labeled probe. Integration of extraction and detection is desirable because it minimizes sample handling, which can lead to sample contamination and degradation which is especially important when handling nucleic acids. The model was constructed as a nucleic acid hybridization sandwich configuration (**Figure 2.2**) with biotinylated [dT]<sub>20</sub> tethered to the NAv modified fiber surface followed by the addition of simulated mRNA tagged with a polyA tail, then probing with a complementary fluorophore

labeled oligonucleotide. The simulated mRNA was designed with oligonucleotide sequences coding for a region of either TNF- $\alpha$  or  $\beta$ -actin specific mRNA sequences including a [dA]<sub>20</sub> tail as a stand-in for the complete mRNA.



By using the model system described above, simulated TNF- $\alpha$  (**Figure 2.3A**) and  $\beta$ -actin mRNA extraction and fluorescence detection was successfully integrated (**Figure 2.3B**) directly on the fibers. Fiber tips were modified by serially spinning through NAV and biotinylated [dT]<sub>20</sub> followed by treatment with different amounts of either simulated TNF- $\alpha$  or  $\beta$ -actin; bound simulated TNF- $\alpha$  and  $\beta$ -actin were then detected via complementary FAM and Texas Red labeled probes. The two probes were linked to spectrally separate fluorescent dyes (FAM and Texas Red) to allow independent detection of both TNF- $\alpha$  and  $\beta$ -actin, and detected

via their respective dye's different emission. The LOD for on-fiber detection of TNF- $\alpha$  and  $\beta$ -actin was calculated to be 260 and 140 fmol respectively. **Figure 2.3** also shows very low fluorescence associated with tips not exposed to the simulated mRNA oligos.



Instead, those tips were treated with 1 uM of randomly generated oligo. An ideal assay would allow detection of multiple analytes in a single assay. The advantage of using fluorescence detection is the capability to be able to detect multiple mRNAs on a single fiber tip to maximize throughput of measuring multiple gene sequences simultaneously. Using the same model system, fluorescence was detected for both simulated β-actin and TNF-α on the same tip. Both β-actin and TNF-α were diluted together where β-actin was concentrated 10 fold more than TNF-α. The fibers were modified with NAv and biotinylated [dT]<sub>20</sub> followed by the

addition of the TNF-α/β-actin mixture and lastly a mixture of the complimentary fluorescent labeled probes. **Figure 2.4** demonstrates that fiber tips can be multiplexed still yielding low LOD of 140 and 90 fmol for β-actin and TNF-α respectively. As many as ten genes could be detected in one tip if individual detection probes were labeled with



different quantum dots with individual emission wavelengths<sup>[146]</sup> Another advantage of microfluidics is the ability to use small volumes. The fibers, when packed together into the capillary tube make narrow channels a few tens of micrometers wide. **Figure 2.5** shows that we can still extract and probe the simulated mRNA using 10  $\mu$ l volumes with 400 fmol LOD for both TNF- $\alpha$  and  $\beta$ -actin.

## Conclusion

An inexpensive, simple, low-tech fluidic fiber solid phase was successfully demonstrated for integrated extraction and detection of nucleic acids using PP C-CP fibers with 100s of fmol LOD using fluorescence detection. The speed is notable where it only takes 1 min per centrifuge spin for each molecular addition. Hence, the entire process (NAv adsorption, tethering of [dT]<sub>20</sub>, mRNA capture, and detection probe hybridization) can take as little as six minutes outside of pipetting and image collection. The PP also has minimal non-specific binding of the DNA oligos in the presence of PBST. The method requires only an imaging apparatus and centrifuge, and is therefore likely to be of use in point of care or near point of care applications. For future directions to reduce costs and data acquisition time, we envision adaptation to visual on-fiber detection by substitution of fluorescent dyes used here for visible dyes (blue dextran) or gold nanoparticles<sup>[80]</sup> This data, together with other publications using C-CP fibers, suggest that the fibers can be used as a platform for a variety of other selective extraction of proteins and metals from complex biological fluids and matrices.

## CHAPTER THREE

### COMPARING MODIFIED CAPILLARY-CHANNELED POLYMER FIBERS FOR SELECTIVE PROTEIN EXTRACTIONS AND RECOVERY WITH STREPTAVIDIN CONJUGATED MAGNETIC BEADS

Protein-protein interactions inside a cell act as cues that mediate cellular function and gene expression<sup>[147-150]</sup>. The most common tools used for confirming and discovering specific protein-protein interactions inside cells are yeast two hybrid (Y2H)<sup>[150, 151]</sup>, phage display<sup>[152, 153]</sup>, tandem affinity purification<sup>[151]</sup>, and pull-down or immunoprecipitation assays.

A pull-down assay is another *in-vitro* affinity purification method used to help confirm or discover stable protein-protein interactions from a cell lysate. Proteins are purified and/or isolated out of solution (usually cell lysate) by affinity to antibodies (for immunoprecipitation and co-immunoprecipitation assays) or other proteins (for pull-downs assays) immobilized to a solid support. The gold standard solid phases used for these assays (especially for small scale) are micron-sized porous agarose<sup>[49, 154]</sup> or non-porous magnetic beads conjugated to specific affinity tags to immobilize proteins and antibodies to the surface<sup>[154, 155]</sup>.

A pull-down assay confirms or discovers specific protein-protein interactions by selective extraction using biomolecular recognition and requires a solid support that can be easily modified for specific functionality. The solid support is usually modified with a protein, known as the (“bait”), which is known to interact and bind specifically with high

affinity to the target protein (“prey”) in a complex mixture (eg. lysate). “Bait” proteins are immobilized to a modified solid support via an affinity tag. Fusion of a glutathione-S-transferase (GST) or multiple histidines are standard “bait” protein affinity tags used for many protein pull downs<sup>[49, 156, 157]</sup>.

Beads have advantages of high binding capacity due to either increased surface area of their porous structure such as the case for agarose beads, or an effectively small surface to volume as is the case for non-porous magnetic beads. Another advantage is the ease of use for small scale, high throughput, and parallel measurement. However, binding can be limited by diffusional mass transfer, which can require long equilibration times for dilute proteins especially in viscous solutions such as lysate. Protein pull-downs could be performed more rapidly with higher capture efficiency using conditions that promote mass transfer, such as microfluidics for higher binding efficiency and recovery.

The C-CP fiber packed micro spin column do not compare to the surface area of porous bead packed columns, but Marcus and coworkers have found that the nonporous nature of the fibers allow for more convective mass transfer (C-term) of proteins, meaning potentially more surface area exposed in a dynamic situation, promoting rapid mass transfer and higher binding efficiency compared to beads for pull-down assays for dilute proteins in lysate.

For our work, wound PP C-CP fibers packed into 0.8 mm FEP tubing. The resulting “fiber bundle” was then adapted to be used as a mini-spin column by cutting into 0.5 cm pieces of the 30 cm “fiber bundle” and fitted onto the end of a micropipette tip. In this case, the pipet tip served as a reservoir for fluids to be passed through the fiber during

centrifugation. The fluidic fiber format has other advantages. For example, the polymer nature of C-CP fibers allows them to be stable at many different pHs and inert to most aqueous solvents. The resulting microfluidic assay platform is inexpensive, easily constructed, and easy to use since most of the equipment is commonly found in most labs, such as a centrifuge, micropipette tips, and centrifuge tubes. Not too mention, several assays can be performed in parallel. The numbers of assays are just limited by the number of slots available in the centrifuge rotor.

The study here demonstrates a protein pull-down assay using PP C-CP fiber spin columns. Proteins bound on the platform will be either be eluted off the solid phases and analyzed by SDS-PAGE or probed by fluorescent labeled proteins for on-fiber detection. As a proof of principle assay, we demonstrated platform performance using the anthrax toxin receptor protein capillary morphogenesis protein 2 (CMG2) as the “bait” and anthrax protective antigen (PA) as the “prey” protein. *In vivo*, PA binds to CMG2 and mediates entry of anthrax toxin into the cell<sup>[158, 159]</sup>. Pull-down performance was compared to that obtained in a similar assay using streptavidin coated magnetic beads. We hypothesized that the fibers will outperform the beads in cell lysate, due to the mass transfer advantages of fluidic flow in the fiber channels.

## **Experimental Section**

### Materials and Methods

NAv was purchased from Thermo Scientific and hydrated with doubly deionized water (ddH<sub>2</sub>O) into a stock of 2 mg/ml. NAv was then diluted into PBS (Cellgro) for experiments. HEPES buffer saline (HBS) was used to dilute biotinylated-CMG2 and PA.

HBS was prepared by dissolving NaCl (150 mM), HEPES (50 mM), MgCl<sub>2</sub> (1 mM) in ddH<sub>2</sub>O, and the adjusting the pH up to 7.9 with 10 M NaOH. A 1% BSA (Fraction V, OmniPur) stock solution was made by dissolving BSA into PBS. The final blocking solution was further diluting BSA into PBS to .1% along with Tween-20 (Rockland) for a total of .1% BSA (w/v) and .01% Tween-20 (v/v) in the blocking solution. NAv-DyLight (Pierce) was hydrated into a 1 mg/ml stock with ddH<sub>2</sub>O. Hydrophilic streptavidin magnetic particles (4 mg/ml) were purchased from New England Biolabs. SDS-PAGE Express pre-cast gradient gels (4-20%) were purchased from GenScript.

#### Fiber bundle and spin column construction

The PP C-CP “fiber bundles” were constructed similarly to the method listed above

#### Protein preparation and labeling

Protein preparation and labeling are explained more in detail elsewhere<sup>[160, 161]</sup>. Maleimide functionalized Alexa Fluor 546 and biotin were added to PA and CMG2<sup>C40</sup> respectively as described previously<sup>[161]</sup>.

#### Functionalizing fibers with NAv-DyLight

The first round of experiments was to show NAv-Dylight binding and compared it to bare fiber columns with no NAv-Dylight spun through and another set treated first with a molar equivalent of BSA. Each fiber column was functionalized by serially spinning through 200 ul PBS, 400 ul NAv-DyLight (.05 mg/ml), and 1 ml PBS. The control group of bare fiber columns had used 400 ul PBS in place of NAv-Dylight and the other set was treated with a molar equivalent of BSA followed by treatment with NAv-Dylight.

A second experiment wanted to determine NAv stability on the fibers in the presence of blocking solution and its individual components. This was done by visualization and monitoring fluorescence using NAv-Dylight. Both bare fiber controls and NAv-Dylight treated fibers were serially treated with the next step of 1 ml of .1% BSA, .1% BSA with .01% Tween-20, .01% Tween-20, and 1 ml PBS followed by a 1 ml PBS wash. The fluorescence from the fibers were measured and imaged on-fiber before and after treatment with blocking solution using the fluorescence microscope. The fluorescence on the fiber after treatment with blocking solution and its individual components was the calculated as a ratio of the initial fluorescence of NAv-Dylight before treatment with the blocking solution.

#### Biotinylated-EGFP binding experiments

To measure if biotinylated molecules bound to the fiber as well as amount, biotinylated-EGFP was donated as a generous gift from Dr. George Chumanov to be used for easily visualization and quantification of biotin bound to fibers and streptavidin conjugated magnetic beads.

The first experiment was to determine biotinylated-EGFP binding to the NAv modified fiber tips. For this first NAv was added to the fibers (.05 mg/ml, 400 ul), followed by 1 ml PBS wash, and the final biotinylated-EGFP solution (50 pmol, 400 ul), followed by a final 1 ml PBS wash. Two other control groups had buffer used in place of biotinylated-EGFP and the NAv modification step. The EGFP fluorescence was then visualized on the fibers using a fluorescence microscope.

The second experiment used biotinylated-EGFP to measure the amount of biotin binding (pmol) to fibers and streptavidin magnetic beads by measuring and comparing the fluorescence of the unbound biotinylated-EGFP in the fiber flow through and post incubated bead solution to an initial EGFP fluorescence value that was added to both fibers and beads. The fluorescence was measured using a plate reader (Tecan instruments). The values left over were then taken as a percentage of the initial fluorescence and calculated to its corresponding pmol value based off of 100% unbound EGFP fluorescence being 50 pmol of biotinylated-EGFP. The values obtained from this experiment were used later on to adjust and normalize the amount of streptavidin magnetic beads to biotin binding.

#### Data acquisition for fluorescence detection

Fluorescence from the fiber columns was imaged with fibers still packed into the FEP tubing at 2X magnification, using a fluorescence microscope (Olympus). The excitation and emission filters were set to 575 ex /624 em for Dylight and Alexa Fluor 546 fluorescence detection. Filters were set to 494 ex/531 em for EGFP on fiber fluorescence measurements.

#### Probing CMG2 with PA-AlexaFluor 546

All fibers (in triplicate) were treated with 200 ul PBS, 400 ul NAv (.05 mg/ml), 1 ml blocking solution, 1 ml PBS, 400 ul biotin-CMG2 (50 pmol) (Buffer for negative control), 1 ml HBS, 400 ul PA-AF546 (various concentration) and 1 ml HBS. The fibers were then imaged using the fluorescence microscope.

#### Fiber and bead comparison of PA pull down from lysate

Four fiber bundles (.5 cm) were assembled into the tip/spin column apparatus where one of the four was a control with no biotin-CMG2. Each bundle was to receive periplasmic lysate but at different volumes (100 ul, 1 ml, and 10 ml). All fiber bundles were functionalized with 200 ul PBS, 400 ul NAv (.05 mg/ml), 1 ml blocking solution, 1 ml PBS, 400 ul biotin-CMG2 (HBS for control), 1 ml HBS, lysate (various volumes), and 1 ml HBS. The control fiber bundle received 1 ml of periplasmic lysate. Four eppendorf tubes each contained 60 ug of hydrophilic streptavidin coated magnetic beads. The amount of beads was normalized to bind the same amount of biotin as the fibers (experimentally determined to be 11 pmol). First, the beads were washed separately in their respective tubes with 200 ul PBS (incubated 1 min. X 3). The functionalization method was similar to the fibers where each tube of beads was to receive the lysate at different volumes with one of the four as a control with no biotin-CMG2. All 4 tubes of beads were functionalized with the same volumes and reagents as fibers minus the NAv since they were already functionalized with streptavidin. All incubations for volumes less than 1 ml were 1 min and 2 min for 1 ml except 20 min incubation for the 10 ml lysate step. Both fibers and beads had the proteins eluted off with 30 ul of 5% SDS (in PBS). The 30 ul elutions were analyzed by SDS-PAGE and loaded onto a gel along with a 1 ug PA standard and molecular weight ladder.

#### SDS-PAGE gel

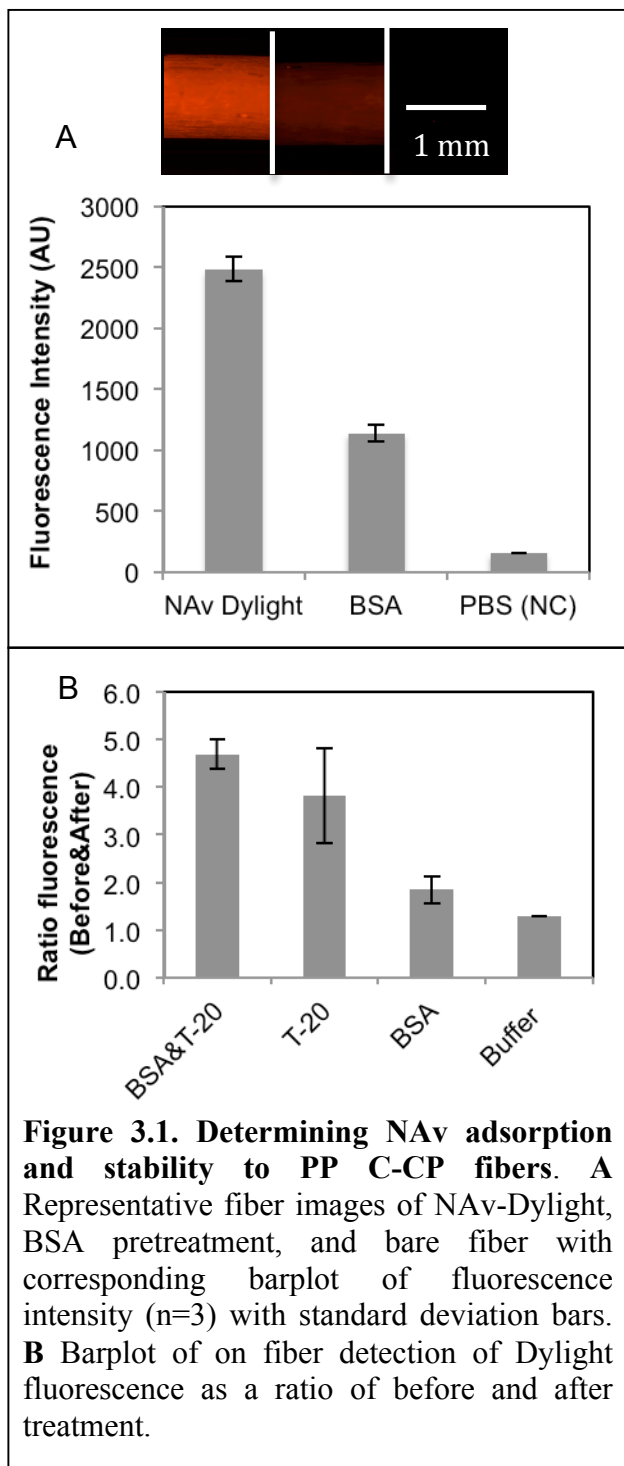
A GenScript Express 10 well pre-cast gradient gel (4-20%) was used for analysis. The 1X running buffer was made by dissolving Tris base (6.06 g), MOPS (10.46 g), SDS (1.0 g), and disodium EDTA (.30 g) diluted up to 1000 ml with deionized water. The voltage



was constant at 140 V. The loading buffer was .3125 M Tris-Cl pH 6.8, 10% SDS, 50% glycerol, with 2-mercaptoethanol. Samples were loaded by adding 30 ul of elution plus 6ul of 5X loading buffer. The samples, including the PA sample were heated at 95° C for 5 min before loading.

## **Results and discussion**

Most pull-downs have a “bait” protein tethered to a solid support that is specific to a particular protein (“prey”). The “bait” protein is either recombinantly fused to another protein or tagged to a molecule for it to be tethered to the surface of the solid phase. Pull-downs can be performed on either a large scale, which then chromatography columns packed with microbeads coated with the “bait” protein or just using the beads as suspensions in tubes for small-scale reactions. Small scale makes it easier for higher throughput for parallel assays. Beads are available pre-conjugated to either protein A or G to immobilize the “bait” IgG for immunoprecipitation assays or tethered to a glutathione protein to immobilize glutathione-S-transferase fused “bait” proteins<sup>[154]</sup>. This helps for selective purification and to not to hinder the binding activity of the “bait” towards specific protein binding partners. To not hinder the “bait” (CMG2) binding activity towards PA, CMG2 was tagged with a biotin molecule. To tether it on the surface, NAv was physically adsorbed to the PP fiber surface. Adsorbed NAv on the fiber surface will then serve to tether any biotinylated recognition moiety, via the tight NAv-biotin interaction. PP fibers have been previously shown to interact with proteins via strong hydrophobic interactions. They have also been used in reverse phase chromatography where the only way to remove proteins from the PP surface was by



increasing the hydrophobicity of the mobile phase. Both these results indicate that the proteins physically adsorb to the PP surface and by way of hydrophobic interactions. However, there are no studies showing NAv adsorption to PP C-CP fibers. To demonstrate that NAv adsorbs to the hydrophobic PP fiber surface, experiments were conducted with fluorescently labeled NAv (NAv-Dylight). Images and the fluorescence of the Dylight were measured directly from each fiber column still packed into the FEP tubing using a fluorescence microscope. Using NAv-Dylight showed that BSA has to adsorb to the fibers because fibers treated with BSA before NAv-Dylight had a

significantly lower fluorescence than fibers that were treated with just NAv-Dylight with no BSA pretreatment. Fluorescent images of fibers treated with NAv-Dylight show

significant fluorescence versus a negative control (NC) of bare (no protein) treated fiber (**Figure 3.1A**) indicating that NAv adsorbs to the PP surface and from the fluorescence from the fiber shows an even coating. NAv stability was tested against the individual and combined components of the blocking solution (Tween-20 and BSA) compared to a control of just PBS. Upon the addition of the blocking step NAv-Dylight fluorescence decreases significantly compared to the fiber image before the addition of the blocking solution (**Figure 3.1B**). The control (PBS treated fibers) showed the least change while Tween-20 decreased Dylight fluorescence the most. This suggests that both BSA and Tween-20 compete off NAv-Dylight. BSA contains hydrophobic groups, and the Marcus and coworkers have showed BSA interaction on PP fibers by physical adsorption. Tween-20 is a non-ionic surfactant with hydrophobic groups and a long hydro-carbon chain. Both then have the possibility of binding to the PP surface, which is why they were originally thought to be used as blocking agents. However, they seem to have stronger interactions with the PP fibers and compete off the NAv. Therefore, different blocking agents or concentrations of the BSA and Tween-20 need to be used so as not to compete off the NAv. However it is still uncertain whether they strip off weakly bound interactions and the stronger bound protein remain at the surface, or if it is stripping off weakly bound Dylight dye. Protein activity may be reduced by the conformational changes when adsorbed to a surface. Therefore, as its conformation changes, then its binding activity can decrease. Since PP is hydrophobic, NAv would probably have to unfold to expose some of its hydrophobic patches and bind to the surface of PP with

multiple van der Waals interactions. This could interfere with its binding activity towards biotin.

	pmol biotinylated-GFP	Stdev (+/- pmol)
Initial	50	N/A
Bound to fibers	11	3
Bound to beads	36	2

**Table 1. pmol of biotinylated-GFP bound to fibers and streptavidin magnetic beads (n=3)**

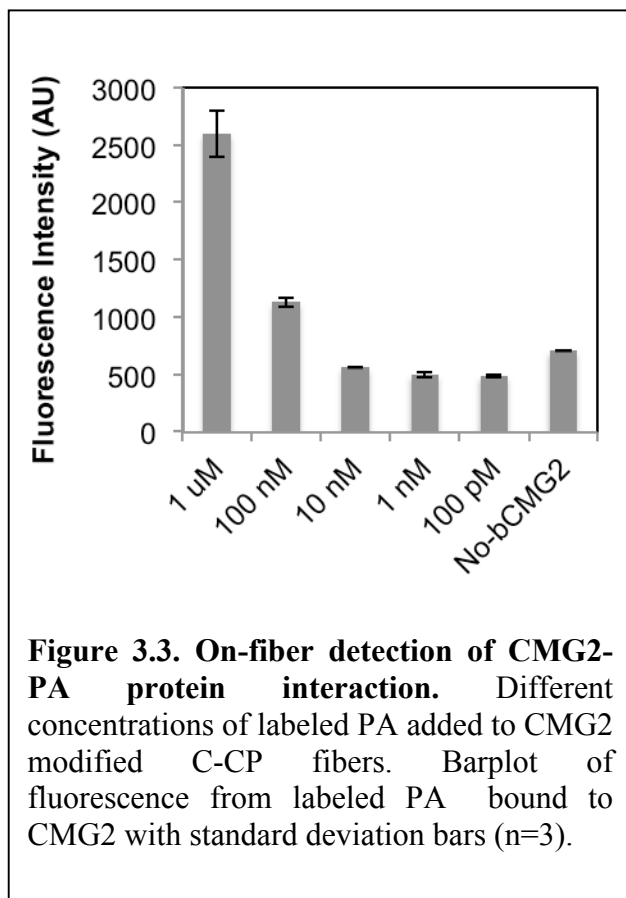


**Figure 3.2 NAv modified C-CP fibers binds biotinylated EGFP. Top** Table of the pmol bound to both fibers and beads (n=3). **Bottom** Representative fiber images of EGFP fluorescence. Far left panel was treated with NAv and biotin-EGFP, middle panel only NAv, and right panel biotin-EGFP.

To test if NAv was still active and bound biotin after adsorption, EGFP was tagged to a biotin molecule to easily visualize the biotin binding and to normalize the amount of beads needed to compare to the fibers. The fluorescence of EGFP was measured on the fiber tips using a fluorescence microscope. The presence of fluorescence on the fiber would then indicate that biotin bound to the NAv. The data showed (**Table 3.1**)

that biotin-EGFP bound to the NAv. Bead quantity was chosen with equivalent biotin-binding based on the results of an experiment to determine biotin binding between fiber columns and beads. Biotin binding was compared between streptavidin conjugated magnetic beads and NAv modified fiber tips. The amount bound was determined by measuring fluorescence of biotin-EGFP added to fibers and beads (the fluorescence value was considered 100%) and measuring the fluorescence from the flow through of the fibers and the biotin-EGFP after incubation with the magnetic beads which the fluorescence was taken as a percentage of the initial fluorescence measurement. The

magnetic beads were added to compare the total surface area of the fibers, and showed that more biotin-EGFP bound to the magnetic beads than the fiber tips (**Figure 3.2**).



Fluorescence has its merits in analytical detection because of its sensitivity and low limits of detection since the light that is detected is mostly from the emitting molecule. For this case a model system was designed to selectively extract and detect protein-protein interactions by on fiber fluorescence detection. The “bait” protein (CMG2) was biotinylated and tethered to the NAv modified fiber via biotin-

NAv interaction, and the “prey” PA was tagged with a fluorophore (PA-AF546). Different concentrations of purified labeled PA were spun through the biotinylated-CMG2 coated fibers, and the fluorescence was measured on the fibers using a fluorescence microscope (**Figure 3.3**). Only fibers treated with all three proteins (NAv, biotinylated-CMG2, and PA-AF546) fluoresced compared to negative controls, indicating specific interaction of PA to CMG2 tethered on the fiber surface. This also verifies previous experiments that NAv and biotinylated-CMG2 would have to be both

functionalized near the fiber surface because there was minimal fluorescence seen for fiber columns not treated with either NAv or biotinylated-CMG2. However, most pull-down assays are performed in more complex solutions with other proteins, and non-specific binding goes up the more concentrated the proteins are in the solution. Pull-downs are typically performed in complex solutions such as lysate, in which small quantities of target/analyte protein are presented in high concentrations of other proteins. To demonstrate specific capture of PA by CMG2 in a complex solution, we treated CMG2-modified fiber spin columns with periplasmic lysate from PA-expressing cells. In this case, columns were treated with different volumes of lysate (.1-10 ml), spanning 2 orders of magnitude. Bound proteins were eluted from tips and visualized using SDS PAGE (**Figure 3.4**). Comparison of eluted protein with a PA standard (lane 2) shows that fiber columns capture PA out of lysate. There was much more non-specific binding for the fiber columns treated with 10 ml of lysate. Single NAv subunits were also visualized on the gel for the fiber columns since they matched near 17 kDa on the molecular weight ladder. There was also faint bands seen near 26 kDa for the fiber columns treated with biotinylated-CMG2, but not seen for the control fiber (not treated with biotinylated-CMG2). Also there was not much non-specific binding for volumes added under 10 ml, and minimal PA non specific binding to both fiber column and beads that were not treated with biotinylated-CMG2.

For comparison, similar experiments were carried out using streptavidin-modified magnetic beads. The beads were incubated with lysate for time equivalent to the fiber spin time. As shown in **Figure 3.4**, quantities of PA pulled down out of lysate, onto the

beads, are roughly similar for fiber tips and CMG2-modified beads. There was also much

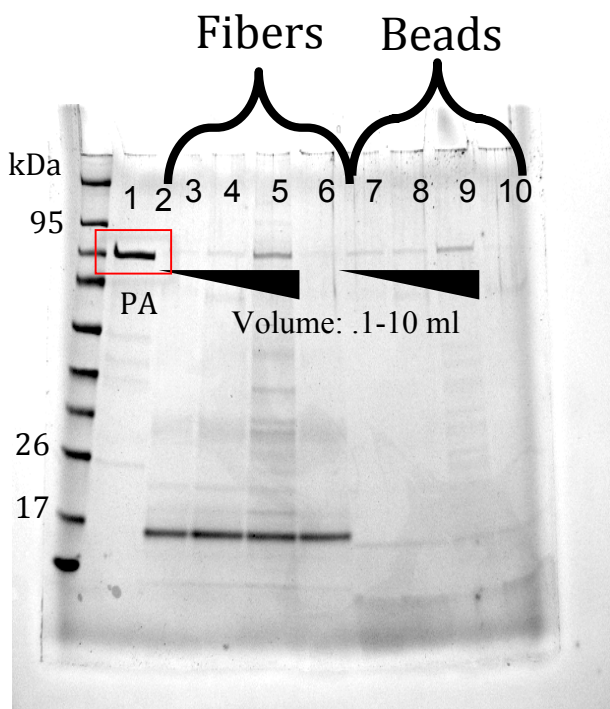
less non-specific binding for the beads

incubated with the 10 ml of lysate.

### Conclusion

It was originally hypothesized that the mass transfer properties of fluidic flow in the fiber channels would perform better with more efficient extraction than diffusion limited assays such as beads based on previous fiber data indicating increased mass transport of proteins for chromatographic separations. However,

in the end, recovery from both fibers and streptavidin magnetic beads were similar as shown from the band intensities in the gel. It is not fully understood as to why that is. This could be due to mass transfer limitations or some sort of kinetic effect concerning the orientation and density of the “bait” CMG2 immobilized onto the fibers. Even though affinity between the



**Figure 3.4. SDS polyacrylamide gel of protein(s) eluted from CMG2-modified fiber bundles and beads.** Fiber tips modified with NAv and biotin-CMG2 were treated with lysate from PA-expressing cells (0.1 mL (lane 3); 1 mL (lane 4); 10 mL (lane 5)). Lanes 7 – 10 reflect corresponding data for NAv-magnetic beads modified with CMG2-biotin. Fibers and beads were normalized for biotin binding to allow comparison of PA capture by equivalent CMG2 concentration in two different formats. Total bound protein was eluted with 5% SDS. The red box (lane 2) indicates 1 ug of PA standard; lanes 6 and 10 reflect eluate from fibers and beads not treated with lysate. Protein ladder in far left lane was used to verify proteins by molecular weight.

biomolecular recognition ligand and protein can be high (as for CMG2 and PA), kinetic effects on the solid phase can make it appear less so. For example, binding has shown to also be dependent upon the orientation and surface density<sup>[162-165]</sup> of the immobilized affinity ligand. The fiber may still have rapid mass transport, but if the “bait” CMG2 was not at the optimal density and configuration, the fluid flowing through the fiber channels might not allow enough time for the PA proteins to stay at the surface to form the necessary bonds. However, depending on the application will also depend on how much protein is actually needed.

It is believed that once these questions are answered, the fibers will be a good material for a selective solid support for small scale immunoprecipitation and pull-down assays. They have already shown to be selective in a variety of complex mixtures, the polymer material makes them chemically stable and resistant to a variety of solvents and pH, they do not dry out, can be reusable, and are stable over long term storage, unlike beads that over time will eventually aggregate.



## CHAPTER FOUR

### CONCLUSIONS

The results in this thesis report successful extraction and on-fiber fluorescence detection of protein (from cell lysate) and nucleic acids (with fmol LOD and multiplex detection) using a neutravidin modified fiber based fluidic platform made from PP C-CP fibers. Protein recovery was similar between fibers and streptavidin conjugated magnetic microspheres.

Widespread use of solid phase extraction is in part due to the development of simple, inexpensive, low-tech, easy to use solid phase configurations. The C-CP spin micro column is simple and cost effective enough to find widespread utility in many different fields for selective solid phase extraction and also find a place in point of care facilities for molecular diagnostics. Research to date also shows the use of other fibers and threads for solid phase extraction and molecular diagnostics assays as an ongoing effort to design simple, compact, fluidic designs from cheap and high abundant materials. The research is relatively new and there needs to be more done to better understand how fibers and threads can effectively be used in these applications. However the literature and the work done using C-CP fibers shows potential for future fiber fluidic sorbent materials and configurations that could be utilized for solid phase extraction and molecular diagnostics.

Although the C-CP fiber material and spin configuration are practical and cost effective, there is room for improvement. Based on this work, it is envisioned that nucleic acid analysis time can be decreased by coupling visual detection instead of fluorescence as well as selectively extracting nucleic acids directly from cell lysate. Future work for

selective protein capture and detection by fundamental studies understanding of protein affinities, orientation, and ligand surface density on the fibers to ensure optimal protein capture efficiency.

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